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NATURAL ANTIMICROBIALS: HEALTHY ALTERNATIVE OF APPLICATION IN FOODS AS PRESERVATIVES

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Abstract: Consumers have sought to adopt a healthier lifestyle by avoiding the use of foods with artificial preservatives. To meet this demand, a solution would be the use of natural preservatives. There are foods that already have natural antimicrobials such as milk (which has lactoferrin), eggs (lysozyme) and crustaceans (chitosan). There are also bacteriocins produced by bacteria to inhibit others, nisin. All these antimicrobials are non-toxic and can be applied to foods as natural preservatives. In this review, we detail each natural antimicrobial and the possible food application that can be directly or through films, coatings, packaging and encapsulation.
Keywords: Health, natural preservatives, shelf life.

INTRODUCTION

Currently, the demand for natural preservatives has grown due to the possible carcinogenic, teratogenic and allergenic effect of artificial preservatives (Vara et al, 2019). (Hayek et al, 2013).

Natural antimicrobials come from natural sources and can be added directly to foods or incorporated into edible films and coatings, so that they are released during food storage and contribute to increasing food shelf life and safety (Hafsa et al, 2016, Lopes and Brandelli, 2017).

New food products with improved quality can be introduced in the market when natural antimicrobial compounds are combined with other natural antimicrobials, and attributing other properties, such as antioxidant activities, can offer organoleptic, technological, nutritional and health benefits (Hugo and Hugo 2015, Jamalabadi et al. al. 2019, Kourkoutas and Proestos, 2020).

LYSOZIME

Lysozyme is an enzyme present in the eggs of birds and in the milk of mammals, it can also be found in saliva, tears, blood serum, some plants and even in certain bacteria and bacteriophages (Wu et al., 2015, Liburdi et al., 2014).

Structurally, lysozyme is a globular protein stabilized with four disulfide bonds that confer high thermal stability to the enzyme (Hamdani et al., 2017). This protein consists of 129 amino acids, has a molecular weight of only 14 kDa, and has an isoelectric point (pI) of 11 (Syngai, and Ahmed, 2019, Sarkar et al., 2020).

The antimicrobial activity of lysozyme is more effective against Gram positive bacteria compared to Gram negative bacteria. Gram-negatives have a lipopolysaccharide layer on the outer layer of the membrane consisting of a physical barrier (Tiwari et al, 2009). Lysozyme is most effective against Gram-negative bacteria when used in combination with nisin and lactoferrin, as these compounds initiate membrane disruption (Barbiroli et al., 2012; Pisoschi et al., 2018).

Chicken egg white lysozyme is reported to be applied as a preservative in fish and fish products, meat and meat products, milk and dairy products, fruits and vegetables (Gyawali and Ibrahim, 2014). Several patents have been issued with the incorporation of lysozyme in food packaging films and application as a coating material for fruit (Hamdani et al., 2017).

Yuceer and Caner (2014) demonstrated an increase in the internal quality of fresh eggs during their storage using chitosan-lysozyme films. Barbiroli et al. (2012) found that lysozyme films with carboxymethylcellulose were effective in decreasing the growth of Listeria on thin slices of meat.

LACTOFERRIN

Lactoferrin is a globular glycoprotein, pink-reddish in color, composed of 692 amino acids, with a molecular weight of 80 kDa and has two symmetrical N and C lobes (Felipe et al, 2017). This protein is present in mammalian secretions such as saliva, tears, gastrointestinal fluids, vaginal fluids, semen, bile and urine. The largest amount of lactoferrin is in milk and colostrum (Rybarczyk et al, 2017).

It is a compound that sequesters iron so it exists in two different forms: halolactoferrin (bound to iron) and apolactoferrin (without iron). It is also able to bind other metals (aluminium, copper, gallium, manganese and zinc) better than iron (González-Chávez et al, 2009). Apolactoferrin has an open conformation, while hololactoferrin is a closed molecule with greater resistance to proteolysis (Adlerova et al, 2008, Jameson et al,1998).

This glycoprotein has antibacterial, antifungal, antiviral and antiparasitic properties (Pawlik et al, 2014). The antimicrobial domain of lactoferrin is located in the N lobe (Baker & Baker, 2009). There are two antibacterial mechanisms: (i) iron sequestration from the environment where the microorganism is and thus, suppresses bacterial growth, (ii) lactoferrin increases cell permeability, inducing cell lysis and death of microorganisms as it is a cationic molecule and interact with lipopolysaccharides, which are anionic (García-Montoya et al, 2012, Messenger et al, 1983, Ortíz-Estrada et al, 2012).

Lactoferrin has been used as an antimicrobial in meats (Pisoschi et al, 2018). Lactoferrin alone or together with nisin improved the microbiological quality of meatballs, leading to a decrease in total aerobic bacteria, coliforms, psychrotrophic bacteria, yeast and molds (Colak et al, 2008). There is a study by Padrão and collaborators (2016) that the bactericidal efficiency of lactoferrin was proven when applied to pork sausage.

LACTOFERRICIN

Peptide derived from the digestion of lactoferrin by the enzyme pepsin (Villalobos-Delgado et al, 2019). The antimicrobial potency of this peptide is much greater than that of the native protein (Farnaud et al, 2003, Gifford et al, 2005). Antimicrobial peptides generally have two common characteristics: cationic charge and a significant proportion of hydrophobic residues (Akalin, 2014).

Although not fully understood, the antibacterial mechanism of lactoferricin is similar to that of lactoferrin, lactoferricin acts by damaging the outer cell wall and destabilizing the cytoplasmic membrane of microorganisms (Sallmann et al, 1999, Yamauchi et al., 1993, Rybarczyk). et al, 2016).

The natural conservation of lactoferricin in cheeses has been proven. Quintieri and colleagues (2012) found lower microbial loads in mozzarella cheese using hydrolyzed lactoferrin compared to unhydrolyzed lactoferrin. Lactoferricin retarded the growth of *Pseudomonas spp*. and coliforms present in Mozzarella cheese in the study by Caputo et al. (2015).

NISIN

Nisin is an antimicrobial peptide of 34 amino acids, synthesized by bacteria: *Lactococcus lactis,* therefore it is a bacteriocin (Arqués et al, 2004, Del Nobile et al, 2012, O'Sullivan, 2012). It acts mainly against Gram positive bacteria, because nisin is cationic, it interacts with the high levels of anionic lipids in the Gram positive membrane resulting in cell disruption (Cleveland et al, 2001).

Another antibacterial mechanism of nisin is the interference with bacterial cell wall biosynthesis, preventing the synthesis of peptidoglycans, a component of membranes, thus causing cell rupture and death (Balciunas et al, 2013, Liao et al, 2018). Although this peptide inhibits many bacterial species such as: *Lactobacillus, Pediococcus, Listeria and Staphylococcus*, this peptide has no antimicrobial activity against gram-negative bacteria, filamentous fungi, yeasts and viruses (Gharsallaoui et al, 2016).

This antimicrobial peptide is the only one with GRAS (generally considered safe) status by the W.H.O. and FDA and is one of the only bacteriocins approved in over 50 countries for use in food preservation.) (Pisoschi et al, 2018).

Nisin has been used as a popular and natural antimicrobial agent in dairy, juice, meat and plant products (Siroli et al, 2019). However, the use of nisin includes some challenges such as uncontrolled antibacterial performance during food storage, sensitivity to environmental stresses, susceptibility to proteolysis and undesirable interactions with food components (Biswaro et al, 2018). To overcome these challenges, nisin has been used together with other antimicrobials (such as chitosan, essential oils, natamycin) or combined with compounds such as pectin or alginate and techniques such as micro or nanoencapsulation (McClements, 2018, Ibarra-Sánchez et al. al, 2019).

Numerous studies prove the antimicrobial activity of nisin combined with other compounds when applied to foods. Microemulsions with nisin and rosemary, thyme and oregano oils showed a bactericidal effect against *B. cereus, L. monocytogenes, Staphylococcus aureus* and *Lactococcus lactis* (Baptista et al, 2019). Encapsulation of nisin with chitosan capsules regulated the release of the compound and maintained the biological activity of the active, solving the problems of enzymatic inactivation of nisin or its interaction with food components (Wu et al, 2017). The bactericidal activity of chitosan showed a synergistic effect with nisin, inhibiting the growth of microorganisms, lipid oxidation and fish protein degradation (Wu et al, 2017, Baptista et al, 2019). Antimicrobial packages with nisin were able to reduce or eliminate microorganisms such as: *L. innocua, S. aureus*, *Bacillus cereus* and *Micrococcus luteus* in cheese or dairy products (Cao-Hoang et al, 2010, Hanušová et al, 2010). Nisin combined with natamycin (another bacteriocin) inhibited the growth of yeasts and molds on olives (Hondrodimou et al, 2011).

CHITOSAN

Chitosan is derived from the deacetylation of chitin (Hamed et al, 2016; Hosseinnejad and Jafari, 2016). Chitin is found in the exoskeletons of crustaceans, molluscs and insects (Cheung et al, 2015, Abdelghany et al, 2019).

This chitin derivative is a cationic, non-toxic, biodegradable and biocompatible polysaccharide (Menazea et al, 2020, Ben-Shalom et al, 2003). Chitosan has antifungal and antibacterial properties, being more effective against Gram-negative than Gram-positive bacteria (Ben-Shalom et al, 2003, Fernandes et al, 2008).

The molecular weight of chitosan plays a crucial role in determining its activity against bacteria (Hayek et al, 2013, Ben-Shalom et al, 2003). Low molecular weight chitosan generally has stronger antibacterial potential compared to high molecular weight chitosan (Hosseinnejad and Jafari, 2016, Abd Elgadir et al, 2015). High molecular weight chitosan forms an impermeable polymeric layer on the bacterial cell surface, blocking the entry of nutrients into the cell, and low molecular weight chitosan penetrates cells and interferes with mRNA and protein synthesis leading to cell death (Hosseinnejad et al. Jafari, 2016, Kumar et al, 2020).

The antibacterial capacity of chitosan also occurs through its positively charged amino groups, which bind to the negatively charged surface of bacterial membranes, causing cell lysis and the death of the microorganism

(Hamed et al, 2016; Perinelli et al, 2018).

Studies show the inhibition of microorganisms caused by chitosan in meats, breads and alcoholic beverages (Lee et al, 2002, Malinowska-Pańczyk et al, 2009, Petrova et al, 2016). Malinowska-Pańczyk and colleagues (2009) found lower total bacterial counts (psychrophilic and psychrotrophic) in chitosan-treated pork compared to control samples. The addition of chitosan inhibited bacterial growth in wheat bread in the work of Lee et al. (2002). In red wine chitosan inhibited yeast: *Brettanomyces bruxellensis* (Petrova et al, 2016).

Chitosan can still be used as food packaging material in the form of films and coatings that can prolong the shelf life of food with the advantages of being biodegradable and non-toxic (Zhou et al, 2021). In addition, chitosan in films and coatings does not affect the sensory properties of packaged food products (Izci et al, 2017, Suresh et al, 2015).

REFERENCES

Abdelghany, A. M., Menazea, A. A., & Ismail, A. M. (2019). Synthesis, characterization and antimicrobial activity of Chitosan/ Polyvinyl Alcohol blend doped with Hibiscus Sabdariffa L. extract. **Journal of Molecular Structure**, 1197, 603-609.

Adlerova, L., Bartoskova, A., & Faldyna, M. J. V. M. (2008). Lactoferrin: a review. **Veterinarni Medicina**, 53(9), 457-468.

Akalın, A. S. (2014). Dairy-derived antimicrobial peptides: action mechanisms, pharmaceutical uses and production proposals. **Trends in Food Science & Technology**, 36(2), 79-95.

Arqués, J. L., Fernández, J., Gaya, P., Nuñez, M., Rodríguez, E., & Medina, M. (2004). Antimicrobial activity of reuterin in combination with nisin against food-borne pathogens. **International Journal of Food Microbiology**, 95(2), 225-229.

Baker, E. N., & Baker, H. M. (2009). A structural framework for understanding the multifunctional character of lactoferrin. **Biochimie**, 91(1), 3-10.

Balciunas, E. M., Martinez, F. A. C., Todorov, S. D., de Melo Franco, B. D. G., Converti, A., & de Souza Oliveira, R. P. (2013). Novel biotechnological applications of bacteriocins: a review. **Food Control**, 32(1), 134-142.

Baptista, R. C., Horita, C. N., & Sant'Ana, A. S. (2020). Natural products with preservative properties for enhancing the microbiological safety and extending the shelf-life of seafood: A review. **Food research international**, 127, 108762.

Barbiroli, A., Bonomi, F., Capretti, G., Iametti, S., Manzoni, M., Piergiovanni, L., & Rollini, M. (2012). Antimicrobial activity of lysozyme and lactoferrin incorporated in cellulose-based food packaging. **Food Control**, 26(2), 387-392.

Ben-Shalom, N., Ardi, R., Pinto, R., Aki, C., & Fallik, E. (2003). Controlling gray mould caused by Botrytis cinerea in cucumber plants by means of chitosan. **Crop Protection**, 22(2), 285-290.

Biswaro, L. S., da Costa Sousa, M. G., Rezende, T., Dias, S. C., & Franco, O. L. (2018). Antimicrobial peptides and nanotechnology, recent advances and challenges. **Frontiers in microbiology**, 9, 855.

Caputo, L., Quintieri, L., Bianchi, D. M., Decastelli, L., Monaci, L., Visconti, A., & Baruzzi, F. (2015). Pepsin-digested bovine lactoferrin prevents Mozzarella cheese blue discoloration caused by Pseudomonas fluorescens. **Food microbiology**, 46, 15-24.

Cao-Hoang, L., Grégoire, L., Chaine, A., & Waché, Y. (2010). Importance and efficiency of in-depth antimicrobial activity for the control of listeria development with nisin-incorporated sodium caseinate films. **Food Control**, 21(9), 1227-1233.

Cheung, R. C. F., Ng, T. B., Wong, J. H., & Chan, W. Y. (2015). Chitosan: an update on potential biomedical and pharmaceutical applications**. Marine drugs**, 13(8), 5156-5186.

Cleveland, J., Montville, T. J., Nes, I. F., & Chikindas, M. L. (2001). Bacteriocins: safe, natural antimicrobials for food preservation. **International journal of food microbiology**, 71(1), 1-20.

Colak, H., Hampikyan, H., Bingol, E. B., & Aksu, H. (2008). The effect of nisin and bovine lactoferrin on the microbiological quality of turkish-style meatball (TEKIRDAĞ KÖFTE). **Journal of Food Safety**, 28(3), 355-375.

Del Nobile, M. A., Lucera, A., Costa, C., & Conte, A. (2012). Food applications of natural antimicrobial compounds. **Frontiers in microbiology**, 3, 287.

Farnaud, S., & Evans, R. W. (2003). Lactoferrin—a multifunctional protein with antimicrobial properties. **Molecular immunology**, 40(7), 395-405.

Felipe, L. D. O., Silva, W. F. D., Araújo, K. C. D., & Fabrino, D. L. (2018). Lactoferrin, chitosan and Melaleuca alternifolia-natural products that show promise in candidiasis treatment. **Brazilian journal of microbiology,** 49, 212-219.

Fernandes, J. C., Tavaria, F. K., Soares, J. C., Ramos, Ó. S., Monteiro, M. J., Pintado, M. E., & Malcata, F. X. (2008). Antimicrobial effects of chitosans and chitooligosaccharides, upon Staphylococcus aureus and Escherichia coli, in food model systems. **Food Microbiology**, 25(7), 922-928.

García-Montoya, I. A., Cendón, T. S., Arévalo-Gallegos, S., & Rascón-Cruz, Q. (2012). Lactoferrin a multiple bioactive protein: an overview. **Biochimica et Biophysica Acta (BBA)-General Subjects**, 1820(3), 226-236.

Gharsallaoui, A., Oulahal, N., Joly, C., & Degraeve, P. (2016). Nisin as a food preservative: part 1: physicochemical properties, antimicrobial activity, and main uses. **Critical reviews in food science and nutrition**, 56(8), 1262-1274.

Gifford, J. L., Hunter, H. N., & Vogel, H. J. (2005). Lactoferricin. **Cellular and molecular life sciences**, 62(22), 2588-2598.

González-Chávez, S. A., Arévalo-Gallegos, S., & Rascón-Cruz, Q. (2009). Lactoferrin: structure, function and applications. **International journal of antimicrobial agents**, 33(4), 301-e1.

Gyawali, R., & Ibrahim, S. A. (2014). Natural products as antimicrobial agents. **Food control**, 46, 412-429.

Hafsa, J., ali Smach, M., Khedher, M. R. B., Charfeddine, B., Limem, K., Majdoub, H., & Rouatbi, S. (2016). Physical, antioxidant and antimicrobial properties of chitosan films containing Eucalyptus globulus essential oil. **LWT-Food Science and Technology**, 68, 356-364.

Hamdani, A. M., Wani, I. A., Bhat, N. A., & Siddiqi, R. A. (2018). Effect of guar gum conjugation on functional, antioxidant and antimicrobial activity of egg white lysozyme. **Food chemistry**, 240, 1201-1209.

Hamed, I., Özogul, F., & Regenstein, J. M. (2016). Industrial applications of crustacean by-products (chitin, chitosan, and chitooligosaccharides): A review. **Trends in food science & technology**, 48, 40-50.

Hanušová, K., Šťastná, M., Votavová, L., Klaudisová, K., Dobiáš, J., Voldřich, M., & Marek, M. (2010). Polymer films releasing nisin and/or natamycin from polyvinyldichloride lacquer coating: Nisin and natamycin migration, efficiency in cheese packaging. **Journal of Food Engineering**, 99(4), 491-496.

Hayek, S. A., Gyawali, R., & Ibrahim, S. A. (2013). Antimicrobial natural products. formatex–microbial pathogens and strategies for combating them: Science. **Technology and Education**, 910-921.

Hondrodimou, O., Kourkoutas, Y., & Panagou, E. Z. (2011). Efficacy of natamycin to control fungal growth in natural black olive fermentation. **Food microbiology**, 28(3), 621-627.

Hosseinnejad, M., & Jafari, S. M. (2016). Evaluation of different factors affecting antimicrobial properties of chitosan. **International journal of biological macromolecules**, 85, 467-475.

Hugo, C. J., & Hugo, A. (2015). Current trends in natural preservatives for fresh sausage products. **Trends in Food Science & Technology**, 45(1), 12-23.

Ibarra-Sánchez, L. A., El-Haddad, N., Mahmoud, D., Miller, M. J., & Karam, L. (2020). Invited review: Advances in nisin use for preservation of dairy products. **Journal of dairy science**, 103(3), 2041-2052.

Izci, L., Ekici, F., & GÜNLÜ, A. (2017). Coating with chitosan film of sea bream (Sparus aurata) fillets: determining shelf life in refrigerator conditions. **Food Science and Technology**, 38, 54-59.

Jamalabadi, M., Saremnezhad, S., Bahrami, A., & Jafari, S. M. (2019). The influence of bath and probe sonication on the physicochemical and microstructural properties of wheat starch. **Food science & nutrition**, 7(7), 2427-2435.

Jameson, G. B., Anderson, B. F., Norris, G. E., Thomas, D. H., & Baker, E. N. (1998). Structure of human apolactoferrin at 2.0 Å resolution. Refinement and analysis of ligand-induced conformational change. **Acta Crystallographica Section D: Biological Crystallography**, 54(6), 1319-1335.

Kourkoutas, Y., & Proestos, C. (2020). Food Preservation: Challenges and Efforts for the Future. **Foods**, 9(4), 391.

Kumar, S., Mukherjee, A., & Dutta, J. (2020). Chitosan based nanocomposite films and coatings: Emerging antimicrobial food packaging alternatives. **Trends in Food Science & Technology**, 97, 196-209.

Lee, H. Y., Kim, S. M., Kim, J. Y., Youn, S. K., Choi, J. S., Park, S. M., & Ahn, D. H. (2002). Effect of addition of chitosan on improvement for shelf life of bread. **Journal of the Korean society of food science and nutrition,** 31(3), 445-450.

Liao, H., Jiang, L., Cheng, Y., Liao, X., & Zhang, R. (2018). Application of nisin-assisted thermosonication processing for preservation and quality retention of fresh apple juice. **Ultrasonics sonochemistry**, 42, 244-249.

Liburdi, K., Benucci, I., & Esti, M. (2014). Lysozyme in wine: an overview of current and future applications. **Comprehensive Reviews in Food Science and Food Safety**, 13(5), 1062-1073.

Lopes, N. A., & Brandelli, A. (2018). Nanostructures for delivery of natural antimicrobials in food. **Critical reviews in food science and nutrition**, 58(13), 2202-2212.

Malinowska-Pańczyk, E., Kołodziejska, I., Murawska, D., & Wołosewicz, G. (2009). The combined effect of moderate pressure and chitosan on Escherichia coli and Staphylococcus aureus cells suspended in a buffer and on natural microflora of apple juice and minced pork. **Food Technology and Biotechnology**, 47(2), 202-209.

McClements, D. J. (2018). Encapsulation, protection, and delivery of bioactive proteins and peptides using nanoparticle and microparticle systems: A review. **Advances in colloid and interface science**, 253, 1-22.

Menazea, A. A., Eid, M. M., & Ahmed, M. K. (2020). Synthesis, characterization, and evaluation of antimicrobial activity of novel Chitosan/Tigecycline composite. **International journal of biological macromolecules**, 147, 194-199.

Messenger, A. J., & Barclay, R. (1983). Bacteria, iron and pathogenicity. **Biochemical Education**, 11(2), 54-63.

Ortíz-Estrada, G., Luna-Castro, S., Piña-Vázquez, C., Samaniego-Barrón, L., León-Sicairos, N., Serrano-Luna, J., & De La Garza, M. (2012). Iron-saturated lactoferrin and pathogenic protozoa: could this protein be an iron source for their parasitic style of life? **Future microbiology**, 7(1), 149-164.

O'Sullivan, D. J. (2012). Exploring the potential to utilize lantibiotic-producing bifidobacteria to create dairy ingredients with increased broadspectrum antimicrobial functionalities yields encouraging results. **Food Technology**, 66(6), 45-50.

Padrão, J., Gonçalves, S., Silva, J. P., Sencadas, V., Lanceros-Méndez, S., Pinheiro, A. C., & Dourado, F. (2016). Bacterial cellulose-lactoferrin as an antimicrobial edible packaging. **Food Hydrocolloids**, 58, 126-140.

Pawlik, A., Sender, G., Korwin-Kossakowska, A., & Oprządek, J. (2014). Immunomodulatory and antimicrobial activity of lactoferrin. **Medycyna Weterynaryjna**, 70(4), 209-213.

Perinelli, D. R., Fagioli, L., Campana, R., Lam, J. K., Baffone, W., Palmieri, G. F., & Bonacucina, G. (2018). Chitosan-based nanosystems and their exploited antimicrobial activity. **European Journal of Pharmaceutical Sciences**, 117, 8-20.

Petrova, B., Cartwright, Z. M., & Edwards, C. G. (2016). Effectiveness of chitosan preparations against Brettanomyces bruxellensis grown in culture media and red wines. **Oeno One**, 50(1), 49-56.

Pisoschi, A. M., Pop, A., Georgescu, C., Turcuş, V., Olah, N. K., & Mathe, E. (2018). An overview of natural antimicrobials role in food. **European Journal of Medicinal Chemistry**, 143, 922-935.

Quintieri, L., Caputo, L., Monaci, L., Deserio, D., Morea, M., & Baruzzi, F. (2012). Antimicrobial efficacy of pepsin-digested bovine lactoferrin on spoilage bacteria contaminating traditional Mozzarella cheese. **Food microbiology**, 31(1), 64-71.

Rybarczyk, J., Kieckens, E., Vanrompay, D., & Cox, E. (2017). In vitro and in vivo studies on the antimicrobial effect of lactoferrin against Escherichia coli O157: H7. **Veterinary microbiology**, *202*, 23-28.

Sallmann, F. R., Baveye-Descamps, S., Pattus, F., Salmon, V., Branza, N., Spik, G., & Legrand, D. (1999). Porins OmpC and PhoE of Escherichia coli as specific cell-surface targets of human lactoferrin: Binding characteristics and biological effects**. Journal of Biological Chemistry**, 274(23), 16107-16114.

Sarkar, S., Gulati, K., Mishra, A., & Poluri, K. M. (2020). Protein nanocomposites: Special inferences to lysozyme based nanomaterials. **International journal of biological macromolecules**, 151, 467-482.

Siroli, L., Camprini, L., Pisano, M. B., Patrignani, F., & Lanciotti, R. (2019). Volatile molecule profiles and anti-Listeria monocytogenes activity of nisin producers Lactococcus lactis strains in vegetable drinks. **Frontiers in microbiology**, 10, 563.

Suresh, P. V., Raj, K. R., Nidheesh, T., Pal, G. K., & Sakhare, P. Z. (2015). Application of chitosan for improvement of quality and shelf life of table eggs under tropical room conditions. **Journal of Food Science and Technology**, 52(10), 6345-6354.

Syngai, G. G., & Ahmed, G. (2019). Lysozyme: A natural antimicrobial enzyme of interest in food applications. In **Enzymes in food biotechnology** (pp. 169-179). Academic Press.

Tiwari, B. K., Valdramidis, V. P., O'Donnell, C. P., Muthukumarappan, K., Bourke, P., & Cullen, P. J. (2009). Application of natural antimicrobials for food preservation. **Journal of agricultural and food chemistry**, 57(14), 5987-6000.

Vara, S., Karnena, M. K., & Dwarapureddi, B. K. (2019). Natural preservatives for nonalcoholic beverages. In **Preservatives and Preservation Approaches in Beverages** (pp. 179-201). Academic Press.

Villalobos-Delgado, L. H., Nevárez-Moorillon, G. V., Caro, I., Quinto, E. J., & Mateo, J. (2019). Natural antimicrobial agents to improve foods shelf life. In **Food quality and shelf life** (pp. 125-157). Academic Press.

Wu, H., Cao, D., Liu, T., Zhao, J., Hu, X., & Li, N. (2015). Purification and characterization of recombinant human lysozyme from eggs of transgenic chickens. **PloS one**, 10(12), e0146032.

Wu, T., Wu, C., Fang, Z., Ma, X., Chen, S., & Hu, Y. (2017). Effect of chitosan microcapsules loaded with nisin on the preservation of small yellow croaker. **Food Control**, *79*, 317-324.

Yamauchi, K., Tomita, M., Giehl, T. J., & Ellison 3rd, R. T. (1993). Antibacterial activity of lactoferrin and a pepsin-derived lactoferrin peptide fragment. **Infection and immunity**, 61(2), 719-728.

Yuceer, M., & Caner, C. (2014). Antimicrobial lysozyme–chitosan coatings affect functional properties and shelf life of chicken eggs during storage. **Journal of the Science of Food and Agriculture**, 94(1), 153-162.

Zhou, D. Y., Wu, Z. X., Yin, F. W., Song, S., Li, A., Zhu, B. W., & Yu, L. L. (2021). Chitosan and Derivatives: Bioactivities and Application in Foods. **Annual Review of Food Science and Technology**, 12, 407-432.